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Comparative evaluation of macro- and microscopic changes in rabbit, cattle, and pig auricular cartilage following exhumation after different *postmortem* intervals

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Abstract

The study evaluated and compared macro- and microscopic changes in the auricular cartilage of rabbits, cattle, and pigs following exhumation at different *postmortem* intervals (PMI). Eight samples corresponding to 0, 5, 10, 15, 20, 25, 30, and 150 days after exhumation were obtained from all rabbit ears, and twelve samples corresponding to 0, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, and 150 days after exhumation were obtained from all cattle and pig ears. The weight loss of the rabbit ear samples remained higher than that of the cattle and pig ear samples at all PMIs. At 60 days, the average weight loss of cattle and pig ear samples did not differ significantly ($p>0.05$). The loss of area of the samples was similar among the three species at most PMIs ($p>0.05$). At 150 days, all cattle samples were completely decomposed, unlike the pig and rabbit samples. Microscopic analysis of the cartilage tinctorial affinity and loss of chondrocyte nuclei and tissue architecture demonstrated the worsening of *postmortem* changes over time, regardless of the animal species. Colonization by fungi and bacteria occurred earlier in the cattle and pig samples. The correlation of time with sample weight loss and cartilage thickness was strong, indicating the potential of these variables as parameters for PMI estimation. Therefore, *postmortem* examination of auricular cartilage can be used to estimate PMI.

Keywords: forensic pathology, *postmortem* interval, decomposition, *postmortem* changes

Introduction

Postmortem interval (PMI) refers to the period between the time of death and the time at which the cadaver is found (2,3). Knowing or estimating PMI can allow the forensic experts to limit the number of suspects by including or excluding individuals, establish the veracity of information obtained from the statements (3), and reconstruct the circumstances in cases of natural death or homicide (27).

Despite the increasing number of studies, PMI estimation remains a major challenge in forensic pathology, due often to the low practical applicability of some trials (11) and long interval between cadaver examination and death (from a few weeks to months) (24).

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Studies of macro- and microscopic changes in different organs and tissues, such as dental pulp (6,23) and eyes (5,13), have demonstrated satisfactory results in terms of PMI estimation in both human and non-human animal cadavers (4,8,17). However, other tissues, such as cartilage, have not been studied as a parameter for PMI estimation (17,18,15). As a tissue that is highly resistant to putrefaction, cartilage warrants further exploration in PMI studies (1).

Cartilage is an avascular tissue with abundant extracellular matrix (ECM), which is synthesized and maintained by chondrocytes. As opposed to other cell types, chondrocytes can survive under low-oxygen and nutrient-deficient conditions. Moreover, chondrocytes exhibit high tolerance of acidosis and hypoxia, and the collagen fibers of the ECM provide protection against saprophytic bacteria. Together, these characteristics render cartilage a promising tissue for PMI estimation, particularly in cases of advanced cadaveric decomposition. Following death, the diffusion of nutrients from the ECM allows chondrocytes to survive for long periods, up to 2 months (1).

Auricular cartilage has been identified as a promising source of tissue for PMI studies, because it is easy to obtain from human cadavers or experimental animal models and it is not very susceptible to common inflammatory and degenerative processes in the articular cartilage. However, only one study has evaluated the breakdown of auricular cartilage to estimate PMI (15).

The ear has been proven a reliable experimental model to study *postmortem* cartilage decomposition (15). However, animal species with varying ear thickness and strength may require different durations to decompose after death, but this has never been studied previously. In addition to animal species, the progression of tissue decomposition is affected by environmental conditions (25). However, these conditions have been little explored in forensic research related to PMI estimation, often being restricted to the measurement of ambient temperature (8) and determination of pH and soil type (17,18,15).

To this end, the aim of the present study was to evaluate and compare the macro- and microscopic changes in the auricular cartilage of cattle, pigs, and rabbits during decomposition under the same conditions of ambient temperature and humidity, soil moisture, and pH at different PMIs.

Material and Methods

Obtaining ears and forming groups

Eight pairs of ears from rabbits, cattle, and pigs of similar breed, sex, and age were collected from eight animals, totaling 16 ears per species. The ears were obtained from slaughterhouses and harvested soon after the animals were slaughtered.

The base, apex, and sides of all ears were discarded to ensure homogeneity in the thicknesses of the samples. Each pair of ears from the same animal was sectioned, in equal parts, each measuring 3 × 2 cm^2, with each sample corresponding to a distinct PMI. All samples, regardless of the animal species, were covered by fur. Eight samples from each pair of rabbit ears were obtained at the indicated PMI (0, 5, 10, 15, 20, 25, 30, and 150 days). The selection of shorter analytical time for rabbit ears was based on previous findings (14). Twelve samples from each pair of cattle and pig ears were obtained at the indicated PMI (0, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, and 150 days).

A group of samples, corresponding to the 8^{th} ear fragment of all rabbits and the 12^{th} ear fragment of all cattle and pigs, was subjected to 150 days of experimentation to monitor the total decomposition of the samples. At time 0, the samples were not buried and were used as the controls for microscopic analysis.

The samples destined for different PMIs were randomly distributed, thus following the principle of randomization of the experimental units (19).

Soil physicochemical analysis

The ear samples were buried on an open ground. Previously, the site was cleared to remove undergrowth and divided into three equal adjacent areas, dedicated to each animal species. The total land area was 22.95 m^2, divided into three sub-areas of approximately 7.65 m^2 per animal species.

For soil physicochemical analysis, 10 samples were collected from each of the three sub-areas, totaling approximately 300 g of soil per area. Ten samples from each sub-area were mixed and homogenized for the analysis of pH, conductivity, organic matter, dispersed clay, particle density, texture, and water content.

In addition, throughout the experimental period, the measurements of soil pH, temperature, and moisture; pluviometric index, and ambient temperature and humidity were performed daily, at the same time in the morning and afternoon, using digital meters.

Sample burial and exhumation

In each of the two sub-areas dedicated to the cattle and pig samples, 11 pits were prepared (20 × 20 × 20 cm in depth, width, and height). In each pit corresponding to each PMI (i.e., 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, and 150 days), eight ear samples were deposited (one sample per animal). Eight samples corresponding to day 0 were not buried.

In the sub-area dedicated to the rabbit samples, seven pits were prepared. In each pit corresponding to each PMI (i.e., 5, 10, 15, 20, 25, 30, and 150 days), eight ear samples were deposited (one sample per animal). Eight samples corresponding to day 0 were not buried.

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The ear samples (3 cm × 2.5 cm) were buried and identified before burying, as each sample was photographed and weighed before and after collection to calculate the weight and area loss during *postmortem* decomposition at each PMI.

The samples from each PMI were buried next to one another. The pits were covered with soil and labeled with species name and harvest day. The pits were only opened on the day of harvest corresponding to each PMI, with the exception of the 150-day pit, which was opened and closed periodically to monitor and record the day of total decomposition of the sample.

Macroscopic analysis

At each PMI, each ear sample from each of the three animal species was subjected to macroscopic analysis. The presence or absence of the following findings was observed: loss of coat (up to 25%, from 25 to 50%, and from 50 to 100%), loss of tissue continuity (up to 25%, from 25 to 50%, and from 50 to 100%), and presence of fungi and cadaveric wax. In each pit, the presence or absence of arthropods and/or annelids was observed. Finally, for each animal species, the frequency (%) of each *postmortem* finding at each PMI was calculated.

Before being buried, the samples were properly identified, weighed on a precision scale, and photographed. After exhuming, the samples were cleaned with a brush to remove the soil, weighed, and photographed again. Sample weight at the end of each PMI was subtracted from the initial weight of the same sample before being buried to determine weight loss (%) during decomposition.

Before and after exhumation, both sides of each ear fragment were photographed with the Canon Powershotsx 530® camera coupled to a Zeiss® stereomicroscope (Lupe) at 2.5× and 4× magnification. The captured images were analyzed using ImageJ 1.52 (The National Institute of Health, USA; http://imagej.nih.gov.ij) to measure the area of the samples and the area covered by coat. The initial value of the sample area and area covered by coat of each fragment before being buried was subtracted from the value obtained at the end of PMI to determine the loss of area and coat (%) during decomposition.

Histomorphometric analysis

Following macroscopic analysis, each ear sample from each PMI was sectioned into three pieces (thickness: ~0.2 mm) to obtain samples from the ends and middle of the fragment, considering that the decomposition process was not uniform throughout the sample. The material was processed through the routine techniques of paraffin embedding, followed by microtomy, to obtain 4-µm-thick histological sections. The histological sections were stained with hematoxylin and eosin. The samples were also stained with the periodic acid-reactive Schiff (PAS) technique to facilitate and confirm the visualization of fungi.

Using the Eclipe E200 light photomicroscope (Nikon®) with 10× and 40× objectives, three images were obtained from each sample; two images were captured from the extremities, and one image was captured from the central region of the sample. The captured areas were enlarged for microscopic analysis. Three images were captured from all samples of the three species at each PMI, including day 0.

On histological sections, auricular cartilage thickness was measured using ImageJ 1.52. Measurements were taken at 30 random points to obtain the mean cartilage thickness for each sample. This analysis was performed on all samples from the three species at each PMI, including day 0. In addition, from the three cartilage images obtained from each sample, the loss of tinctorial affinity, decrease in nuclear material, loss of tissue architecture, and presence of fungi and bacteria were evaluated to determine the frequency of these findings at each PMI and for each animal species.

Statistical analysis

For the percentage loss of weight, coat area, and sample area, a completely randomized design in a split-plot arrangement was used. The design included randomized blocks for the variable cartilage thickness. Tukey's test was performed, and differences were considered significant at $p<0.05$ (19). The correlations among the variables were classified according to the values of the coefficient of determination (R2): strong (0.7–0.9), moderate (0.7–0.5), and weak (<0.5) (17). The R^2 values were used as percentages in the present study.

Results

Soil analysis and environmental parameters

Based on the results of granulometric evaluation, the soil was considered clayey–sandy. Magnesium (2.3 $mg \cdot dm^{-3}$) and calcium (6.4 $mg \cdot dm^{-3}$) content was high, while phosphorus content (7.9 $mg \cdot dm^{-3}$) was moderate. Soil pH was neutral.

The mean ambient temperature during the experimental period was 23.2°C ± 1.57°C (22.0°C ± 1.51°C in the morning and 24.3°C ± 2.03°C in the afternoon). The overall mean humidity was 52.3% ± 3.04% (54.5% ± 3.97% in the morning and 50.3% ± 5.11% in the afternoon). During the experimental period, rain was recorded on the 46th day, with a rainfall index of 10 mm in the studied area.

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Postmortem macroscopic changes in rabbit ears

In the rabbit samples, average percentage weight and area loss increased gradually and significantly up to *postmortem* 30 days. Average percentage loss of weight and tissue area was respectively 19.29% and 7.83% at 5 days and respectively 63.18% and 28.99% at 30 days. However, average percentage loss of coat area exceeded 50% at 5 days and reached 96.88% at 30 days (Table 1). The *postmortem* changes of the rabbit samples at the respective PMIs up to 150 days after exhumation are shown in Figure 1.

Over different PMIs, the loss of tissue continuity in each sample was heterogeneous. Although the sample area was progressively reduced with increased tissue area loss up to 30 days, the loss of continuity of the sample tissue, characterized by the presence of empty spaces, was observed from the 10th day onward only in 12.5% of the samples. At 30 days, 37.5% of the samples showed tissue continuity loss of <50% and 37.5% of the specimens showed tissue continuity loss >50%.

The total tissue decomposition time widely varied among the samples. Two samples each had completely decomposed at 126 and 131 days. Meanwhile, 50% of the remaining samples had not completely decomposed even after 150 days. However, with the exception of one sample, the others had turned friable, with a reduction of over 75% in fragment size from the initial value (Fig. 1).

Whitish coverage, which was proven by microscopic examination to be a fungus, was observed in 50% or more samples from the 15th day. Cadaveric wax was observed in 12.5% of the samples on the 10th and 25th days and in 25% of the samples on the 20th day. Arthropods and annelids were found only in the pit corresponding to 30-day PMI.

Table 1. Mean, standard deviation, and statistical comparison of the percent loss of weight, sample area, and coat area in the rabbit ear samples at different *postmortem* intervals (PMIs), from the 5th to 30th day of exhumation. Means followed by different lower-case letters in the column are different by the Tukey test (p<0,05).

PMI (Days)	Weight loss (%)	Area loss (%)	Hair loss (%)
5	19,29 ± 3,42 a	7,83 ± 3,63 a	50,65 ± 19,32 ab
10	26,30 ± 8,56 a	14,35 ± 3,61 ab	59,18 ± 20,62 a
15	43,45 ± 7,56 b	21,42 ± 11,70 ab	64,33 ± 27,60 ab
20	46,20 ± 4,22 b	23,44 ± 10,17 abc	62,85 ± 28,50 ab
25	57,25 ± 3,94 c	24,79 ± 8,33 bc	87,86 ± 31,32 ab
30	63,18 ± 9,61 d	28,99 ± 10,30 c	96,88 ± 17,72 b

Means followed by different lower-case letters in the column are different by the Tukey test (p<0,05).

Postmortem macroscopic changes in cattle ears

In the cattle samples, the average percentage weight loss increased over the study period, albeit irregularly. The average percentage weight loss was already 20.47% on the 5th day, and it increased significantly until the 25th day to reach 50.60% (p<0.05). From the 40th day onward, the average loss did not differ significantly among the PMIs, reaching 60.48% at 60 days (Table 2).

The average percentage loss of the sample area was 9.77% on the 5th day and increased significantly to reach 33.96% on the 15th day (p<0.05). From the 15th day onward, the average lass did not differ significantly among the PMIs, reaching 28.08% at 60 days, which was statistically similar to the value at 15 days (Table 2).

The average percentage loss of the coat area was 16.06% on the 5th day and increased gradually and significantly to reach 87.31% on the 60th day. The *postmortem* changes of the cattle samples in the respective PMIs up to 130 days after exhumation are shown in Figure 2.

From the 20th day onward, one sample showed up to 25% loss of tissue continuity. This degree of loss remained until the 60th day, with only a progressive increase in the proportion of samples that presented up to 25% loss of tissue continuity. At 60 days, 75% of the samples showed up to 25% loss of tissue continuity, but none showed tissue loss exceeding 25%. The total tissue decomposition time widely varied among the samples (100 days in one sample, 125 days in two samples, 130 days in three samples, and 150 days in two samples) (Fig. 2).

The whitish coverage, which was confirmed by microscopic examination to be a fungus, was observed from the 10th day in 12.5% of the samples. Thereafter, respectively 50% and 75% of the samples presented such coverage on the 15th and 20th days. All samples presented such coverage on the 20th, 40th, and 60th days. Cadaveric wax was observed in 12.5%–25% of the samples on the 10th, 15th, and 20th days. From the 20th day onward, cadaveric wax was no longer observed. Arthropods and annelids were found only in the pits corresponding to 20- and 40-day PMI.

Postmortem macroscopic changes in pig ears

In the pig samples, the average percentage weight loss was 3.91% on the 5th day, and it increased significantly until the 20th day to reach 45.61% (p<0.05). From the 20th day onward, the average percentage weight loss increased but did not differ significantly among the PMIs, reaching 57.24% at 60 days (Table 3).

On the 5th day, the average percentage loss in the area was 12.08%. Thereafter, the percentage loss of area gradually increased; however, value on the 40th day alone (32.22%) significantly differed from that on the 5th day (p<0.05). From the 40th day onward, the average lass did not differ among the

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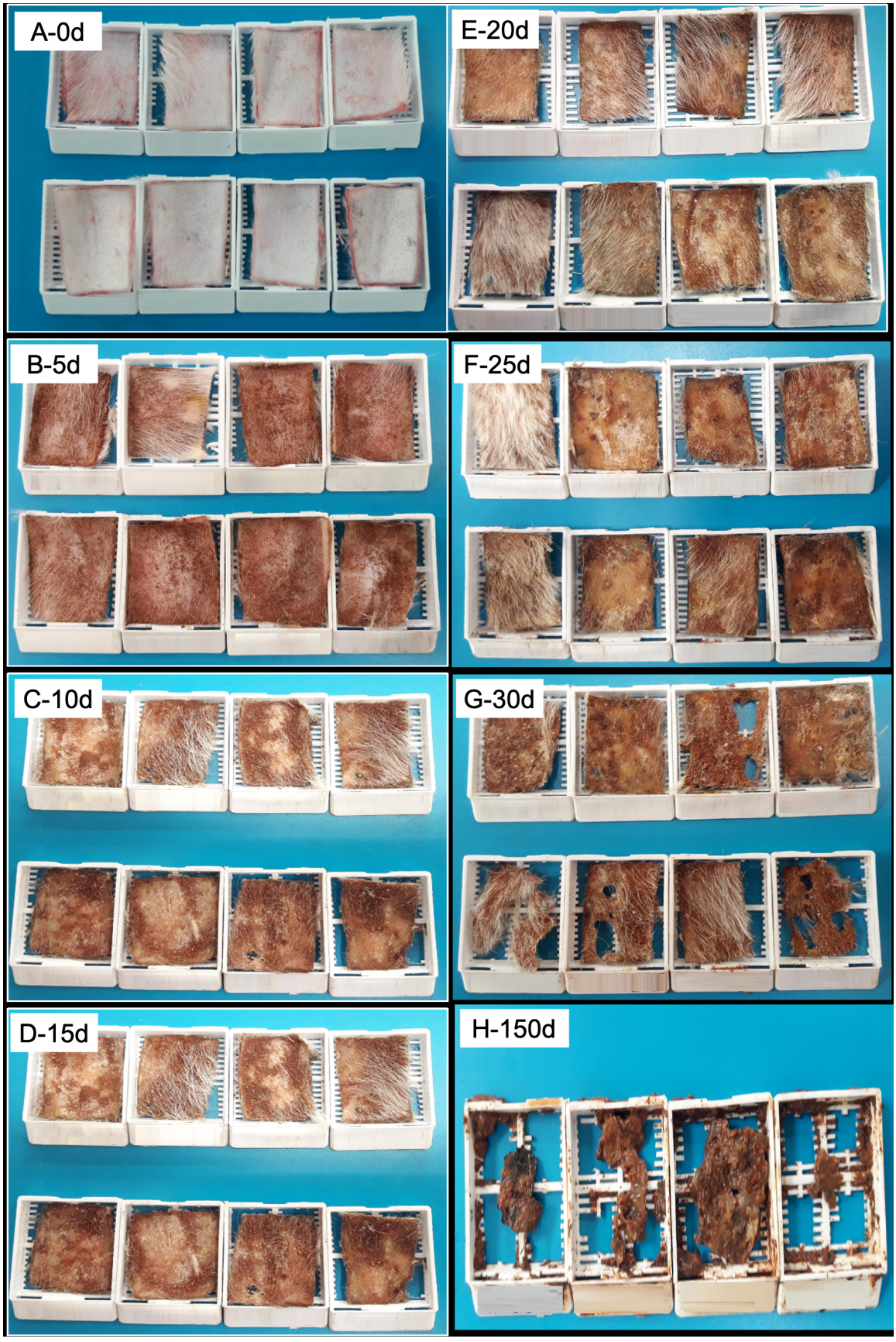

Figure 1. Macroscopic characteristics of the rabbit ear samples at each *postmortem* interval (PMI) after exhumation.

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Table 2. Mean, standard deviation, and statistical comparison of the percent loss of weight, sample area, and coat area in the cattle ear samples at different *postmortem* intervals (PMIs), from the 5th to 60th day of exhumation. Means followed by different lower-case letters in the column are different by the Tukey test (p<0,05).

PMI (Days)	Weight loss (%)	Area loss (%)	Hair loss (%)
5	20,47 ± 5,61 a	9,77 ± 3,98 a	16,06 ± 6,25 ab
10	16,70 ± 5,19 a	11,08 ± 6,86 a	16,93 ± 5,09 a
15	30,58 ± 6,49 b	33,96 ± 8,28 c	26,07 ± 19,05 abc
20	43,75 ± 4,51 c	17,86 ± 14,90 ab	47,40 ± 13,12 bcd
25	50,60 ± 3,82 de	19,11 ± 4,22 b	52,83 ± 23,19 cde
30	47,74 ± 1,98 cd	19,92 ± 3,99 b	56,46 ± 25,51 cde
35	51,87 ± 2,05 e	21,70 ± 8,26 b	58,44 ± 22,41 de
40	62,11 ± 2,84 f	22,32 ± 4,21 b	82,50 ± 15,28 e
50	57,94 ± 3,66 f	26,05 ± 5,56 bc	85,44 ± 21,43 de
60	60,48 ± 6,13 f	28,08 ± 7,08 bc	87,31 ± 22,90 e

Means followed by different lower-case letters in the column are different by the Tukey test (p<0,05).

PMIs, reaching 33.74% at 60 days, which was statistically similar to the value on the 15th day (Table 3).

The average percentage loss of the coat area was already 28.65% on the 5th day. On the 10th day, there was an intense and a significant increase in the loss of the coat area, reaching an average of 63.46% (p<0.05). From the 10th day onward, the average percentage loss of the coat area increased progressively, albeit without statistically significant differences among the PMIs, and reached 90.43% on the 60th day (Table 3). The *postmortem* changes of the pig sample at the respective PMIs up to 150 days after exhumation are shown in Figure 3.

Only after the 20th day, 25% of the samples showed tissue continuity loss of up to 25%. From the 40th day onward, 37.5% of the samples showed up to 25% loss of tissue continuity, which was maintained until the 60th day. At 150 days, no sample had completely decomposed, and the consistency remained firm. However, the samples had reduced in size and completely lost coat (Fig. 3).

The percentage of samples with whitish coverage (fungi) varied greatly throughout the study period. Such coverage first appeared on the 15th day in 75% of the samples. Until the 20th day, all samples showed this change. However, between the 30th and 40th days, 37.5% of the samples were covered by fungi. On the 50th and 60th days, respectively 75% and 87.5% of the samples were covered by fungi.

Cadaveric wax was observed on the 10th, 20th, and 35th days in 37.5%, 12.5%, and 25% of the samples, respectively. At other time points, cadaveric wax was not observed in any sample. Arthropods and annelids were not observed at the time points.

Comparison of macroscopic changes among species

The mean percentage weight loss of the rabbit ear samples was always higher than that of the cattle and pig ear samples, with values being significantly higher at 10, 15, and 30 days (p<0.05). On the 5th day, the weight loss of the rabbit and cattle ear samples was high and statistically similar, with means of 19.29% and 20.47%, respectively, contrary contrast to that of the pig ear samples, with mean of only 3.91% (Fig. 4A and A').

On the 30th day, the average percentage weight loss of the rabbit ear samples (63.18%) was significantly higher than that of the cattle (47.74%) and pig (52.74%) ear samples (p<0.05). Although the average weight loss of the cattle ear samples was significantly lower than that of the pig ear samples at 35 and 50 days, the values were statistically similar at 60 days (Fig. 4A and A').

Among the three animal species, the mean percentage loss of the sample area was statistically similar at most PMIs up to the 30th day, with the exception values on the 15th day, when the cattle samples showed a significantly higher area loss than the rabbit and pig samples. From the 30th to 60th day, the mean percentage loss of the sample area was statistically similar between the cattle and pig samples, with the exception of values on the 40th day (Fig. 4B and B').

The percentage loss of the coat area was significantly higher in the rabbit samples on the 5th day. However, on the 10th, 15th, and 30th days, the loss of the coat area of the rabbit and pig samples was intense and statistically similar, being higher than that of the cattle samples. Between the 35th and 60th days, the mean percentage loss of the coat area was statistically similar between the cattle and pig samples (Fig. 4C and C').

Microscopic postmortem changes in rabbit ears

Microscopic *postmortem* findings of the rabbit ear samples at different PMIs are shown in Figure 5.

On day 0, as expected, the rabbit ear samples were completely preserved, covered by intact hairy skin and with perichondrium and cartilage free of any *postmortem* change. The cartilage was intact and well stained with hematoxylin, with an average thickness of 0.21 mm. Chondrocyte lacunae were large, and binucleate cells and intracytoplasmic lipid droplet vacuoles were frequently observed.

On the 5th day, 75.0% of the samples showed partial loss of cartilage tinctorial affinity for hematoxylin, which was the only finding observed during this period.

On the 10th day, partial and total loss of tinctorial affinity was observed in 79.16% and 16.67% of the samples, respectively. At this time point, *postmortem* microorganisms were also visualized for the first time. Numerous colonies formed by coccoid bacteria were detected in the skin and connective tissue adjacent to the cartilage in 37.5% of the

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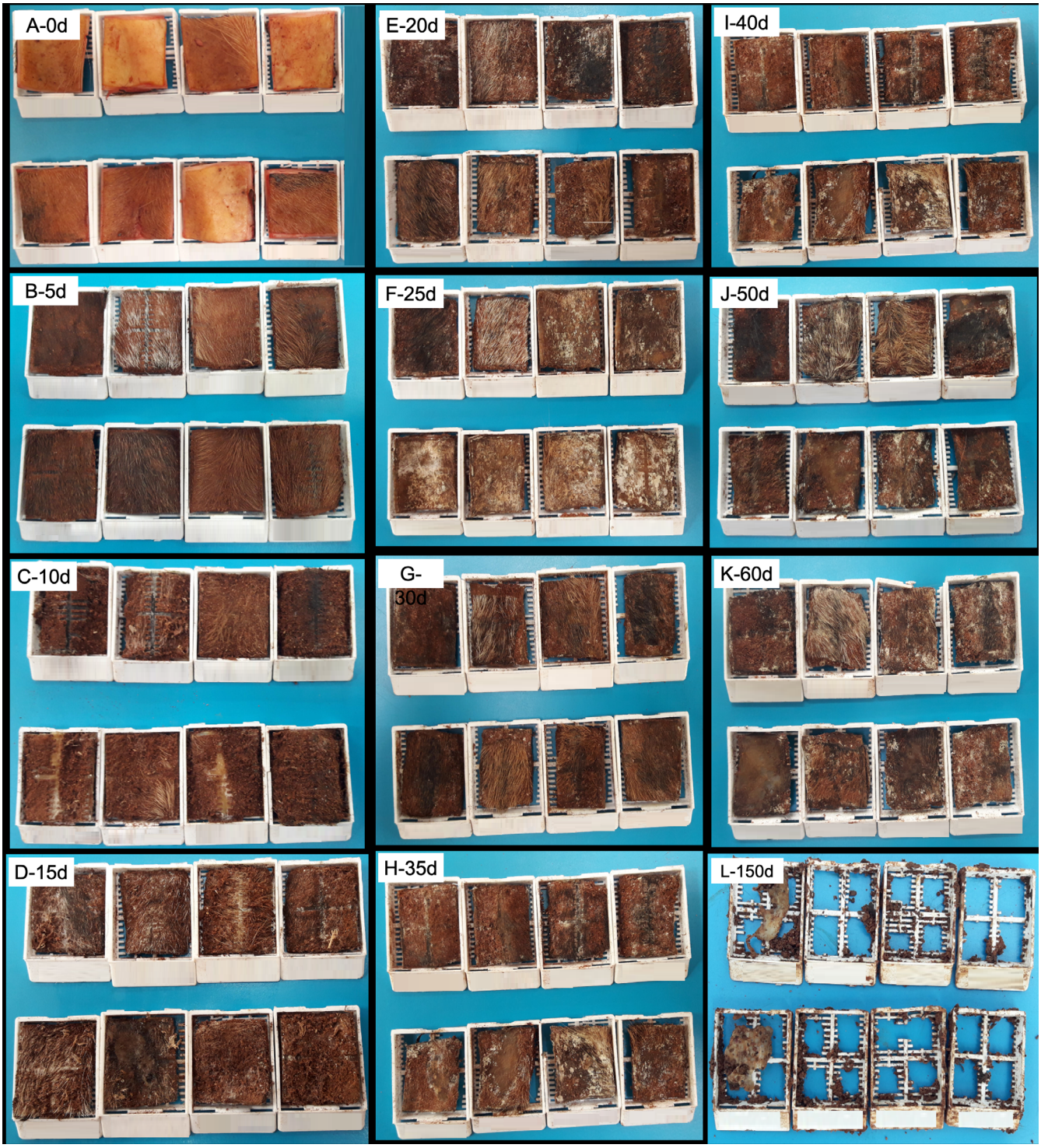

Figure 2. Macroscopic characteristics of the cattle ear samples at each *postmortem* interval (PMI) after exhumation

samples. In 75% of the samples, numerous septate fungal hyphae, with negative images and parallel walls, were visualized in tissues adjacent to the cartilage.

On the 15th days, partial and total loss of cartilage tinctorial affinity was observed in 54.16% and 45.83% of the samples, respectively. Chondrocyte nucleus loss was observed for the first time. Furthermore, partial and total loss of nuclear material was observed 16.67% if the samples each. Moreover, 16.67% of the samples showed partial loss of tissue architecture. Bacteria were observed in the tissues adjacent to the cartilage in 75.0% of the samples and in the cartilage tissue in 37.5% of the samples. Fungal hyphae were observed

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Table 3. Mean, standard deviation, and statistical comparison of the percent loss of weight, sample area, and coat area in the pig ear samples at different *postmortem* intervals (PMIs), from the 5th to 60th day of exhumation. Means followed by different lower-case letters in the column are different by the Tukey test (p<0,05).

PMI (Days)	Weight loss (%)	Area loss (%)	Hair loss (%)
5	3,91 ± 2,08 a	12,08 ± 8,26 a	28,65 ± 10,55 a
10	11,83 ± 2,18 b	14,68 ± 9,02 ab	63,46 ± 27,41 b
15	34,56 ± 3,28 c	21,35 ± 6,50 abcd	63,56 ± 21,53 b
20	45,61 ± 3,83 d	24,55 ± 7,49 abc	74,58 ± 15,33 bc
25	49,33 ± 3,65 de	24,87 ± 10,30 abcd	77,86 ± 16,56 bc
30	52,74 ± 3,10 ef	28,08 ± 10,20 bcd	79,63 ± 12,64 bc
35	57,62 ± 6,31fgh	31,79 ± 11,62 abcd	83,75 ± 20,90 bc
40	63,30 ± 4,12 h	32,22 ± 9,22 cd	85,17 ± 9,98 bc
50	61,50 ± 4,45 gh	32,45 ± 8,57 cd	89,69 ± 6,28 c
60	57,24 ± 3,70 fg	33,74 ± 10,37 d	90,43 ± 27,04 bc

Means followed by different lower-case letters in the column are different by the Tukey test (p<0,05).

in the skin of 91.66% of the samples and in the cartilage of 37.5% of the samples.

On the 20th day, partial and total loss of cartilage tinctorial affinity was observed in 33.33% and 66.67% of the samples, respectively. Total and partial loss of chondrocyte nuclei was observed in 37.5% and 29.17% of the samples, respectively. During this period, partial and total loss of tissue architecture was observed in 16.67% and 37.5% of the samples, respectively. Additionally, bacterial colonies were observed in the skin and adjacent tissues of 79.16% and 20.83% of the samples, respectively. Fungi were observed in tissues adjacent to the cartilage in 87.5% of the samples and in the cartilage tissue in 29.16% of the samples.

On the 25th day, 87.5% and 12.5% of the samples showed total and partial loss of cartilage tinctorial affinity, respectively. During this period, the proportion of samples showing total loss of chondrocyte nuclear material (54.17%) was greater than that of samples showing partial loss (29.17%). Tissue architecture was completely lost in 45.83% of the samples and partially lost in only 12.5% of the samples. Bacterial colonies were observed in the skin and connective tissue (62.5%) as well as in the cartilage (33.33%). Fungal hyphae were observed in the skin and adjacent tissues of 70.83% and 50.0% of the samples, respectively.

Finally, on the 30th day, all samples had completely lost cartilage tinctorial affinity. Total loss of chondrocyte nuclei and tissue architecture was observed in 83.33% of the samples. Partial loss of nuclei was observed in 16.67% of the samples. Bacteria were observed in the adjacent tissues and cartilage in 70.83% and 37.5% of the samples, respectively. Moreover, fungi were observed in the adjacent tissues and cartilage in 87.5% and 75.0% of the samples, respectively.

Although the auricular cartilage had become thin on the 5th day, the reduction in thickness was statistically significant only on the 15th day compared with the value on day 0. From the 15th to 30th day, mean cartilage thickness remained statistically similar among the PMIs (Fig. 5H).

Microscopic postmortem changes in cattle ears

Microscopic *postmortem* findings of the cattle ear samples at different PMIs are shown in Figure 6.

On day 0, the cattle ear samples were completely preserved, covered by intact hairy skin and with perichondrium and cartilage free from any *postmortem* change. The cartilage was intact, being nearly five times thicker than that in the rabbit samples (average thickness, 1.08 mm). The cartilage was well stained with hematoxylin. Chondrocyte lacunae were much smaller than that in the rabbit samples, and the chondrocytes were mononuclear.

On the 5th day, 37.5% of the fragments showed slight loss of cartilage tinctorial affinity. Contrary to the observations in the rabbit samples, bacteria and fungi were already detected in the skin and tissues adjacent to the cartilage in 20.83% and 4.16% of the samples, respectively.

On the 10th day, partial and total loss of cartilage tinctorial affinity was observed in 50.0% and 41.66% of the samples, respectively. During this period, 25% of the samples showed partial loss of chondrocyte nuclei. Bacterial colonies were observed in the skin, connective tissue (29.16%), and cartilage (4.16%). Fungal structures were observed in 29.16% of the samples but were restricted to tissues adjacent to the cartilage.

On the 15th day, similar to the observations in the rabbit samples, the proportion of samples with total loss of cartilage tinctorial affinity (66.67%) was higher than that of samples with partial loss (33.33%). Unlike that in rabbit samples, during this period, many samples (58.33%) had already partially lost chondrocyte nuclei and 8.33% of the samples had completely lost tissue architecture. Bacteria were observed in the skin and adjacent structures of 58.33% of the fragments and in the cartilage of 87.5% of the samples. Fungal hyphae were present in the skin and adjacent tissues, and for the first time in cartilage, in 20.83% and 33.33% of the samples, respectively.

On the 20th day, the number of samples with total and partial loss of cartilage tinctorial affinity was similar to that on the 15th day. However, the number of fragments with partial loss of chondrocyte nuclei (50.0%) was reduced, as justified by the increase in the number of samples with total loss of nuclei (16.66%). The number of samples with partial (20.83%) and total (16.66%) loss of tissue architecture also increased. Bacterial colonies were observed in the skin and cartilage in 45.83% and 33.33% of the samples, respectively.

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Figure 3. Macroscopic characteristics of the pig ear samples at each *postmortem* interval (PMI) after exhumation.

Fungal hyphae were present in the skin and cartilage in 8.33% and 54.16% of the samples, respectively.

On the 25th day, total loss of cartilage tinctorial affinity was observed in 79.17% of the samples and total loss of chondrocyte nuclei and tissue architecture was observed in 62.50% of the samples. Bacteria were observed in the skin (62.50%) and cartilage tissue (75.0%), and fungal hyphae were also present in the skin (16.66%) and cartilage tissue (45.83%).

On the 30th and 35th days, most samples had completely lost cartilage tinctorial affinity, chondrocyte nuclei, and tissue structure. Additionally, similar to the observations at previous time points, bacteria and fungi were observed in the skin, cartilage tissue, and other tissues adjacent to the cartilage.

On the 40th and 50th days, all samples had completely lost cartilage tinctorial affinity and 91.67% of the samples had completely lost chondrocyte nuclear material and tissue

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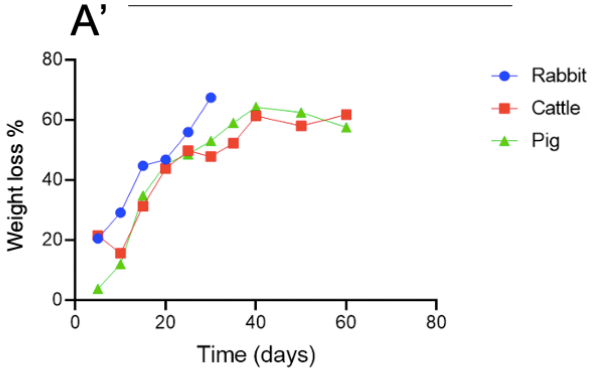
A

PMI (Days)	Species		
	Rabbit	Cattle	Pig
5	19,29 ± 3,42 A	20,47 ± 5,61 A	3,91 ± 2,08 B
10	26,30 ± 8,56 A	16,70 ± 5,19 B	11,83 ± 2,18 B
15	43,52 ± 7,56 A	30,58 ± 6,49 B	34,56 ± 3,28 B
20	46,20 ± 4,22 A	43,75 ± 4,51 A	45,61 ± 3,83 A
25	57,25 ± 3,94 A	50,60 ± 3,82 AB	49,33 ± 3,65 B
30	63,18 ± 9,61 A	47,74 ± 1,98 C	52,74 ± 3,10 B
35		51,87 ± 2,05 B	57,62 ± 6,31A
40		62,11 ± 2,84 A	63,30 ± 4,12 A
50		57,94 ± 3,66 B	61,50 ± 4,45 A
60		60,48 ± 6,13 A	57,24 ± 3,70 A

Means followed by different uppercase letters in line are different by the Tukey test (p<0,05).

A'

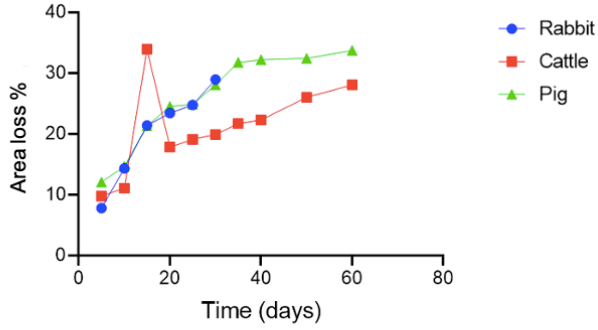


B

PMI (Days)	Species		
	Rabbit	Cattle	Pig
5	7,83 ± 3,63 A	9,77 ± 3,98 A	12,08 ± 8,26 A
10	14,35 ± 3,61 A	11,08 ± 6,86 A	14,68 ± 9,02 A
15	21,42 ± 11,70 B	33,96 ± 8,28 A	21,35 ± 6,50 B
20	23,44 ± 10,17 A	17,86 ± 14,90 A	24,55 ± 7,49 A
25	24,79 ± 8,33 A	19,11 ± 4,22 A	24,87 ± 10,30 A
30	28,99 ± 10,30 A	19,92 ± 3,99 A	28,08 ± 10,20 A
35		21,70 ± 8,26 A	31,79 ± 11,62 A
40		22,32 ± 4,21 B	32,22 ± 9,22 A
50		26,05 ± 5,56 A	32,45 ± 8,57 A
60		28,08 ± 7,08 A	33,74 ± 10,37 A

Means followed by different uppercase letters in line are different by the Tukey test (p<0,05).

B'



C

PMI (Days)	Species		
	Rabbit	Cattle	Pig
5	50,65 ± 19,32 A	16,06 ± 6,25 B	28,65 ± 10,55 B
10	59,18 ± 20,62 A	16,93 ± 5,09 B	63,46 ± 27,41 A
15	64,33 ± 27,60 A	26,07 ± 19,05 B	63,56 ± 21,53 A
20	62,85 ± 28,05 A	47,40 ± 13,12 A	74,58 ± 15,33 A
25	87,86 ± 31,21 A	52,83 ± 23,19 A	77,86 ± 16,56 A
30	96,88 ± 17,72 A	56,46 ± 25,51 B	79,63 ± 12,64 A
35		58,44 ± 22,41 A	83,75 ± 20,90 A
40		82,50 ± 15,28 A	85,17 ± 9,98 A
50		85,44 ± 21,43 A	89,69 ± 6,28 A
60		87,31 ± 22,90 A	90,43 ± 27,04 A

Means followed by different uppercase letters in line are different by the Tukey test (p<0,05).

C'

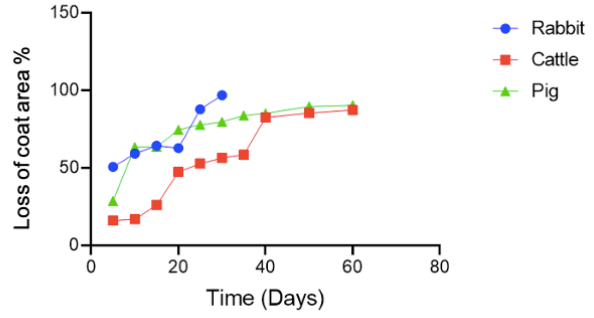


Figure 4. Comparation between species on weight loss: A) Mean and standard deviation of the percent loss of weight among the rabbit, cattle, and pig ear samples at different *postmortem* intervals (PMIs), from the 5th to 60th day of exhumation. Means followed by different lower-case letters in the line are different by the Tukey test (p<0,05), A') Graphic characterization of the percent loss of weight among the rabbit, cattle, and pig ear samples at different *postmortem* intervals (PMIs), from the 5th to 60th day of exhumation, B) Mean and standard deviation of the percent loss of sample area among the rabbit, cattle, and pig samples at different *postmortem* intervals (PMIs), from the 5th to 60th day of exhumation. Means followed by different lower-case letters in the line are different by the Tukey test (p<0,05), B') Graphic characterization of the percent loss of sample area among the rabbit, cattle, and pig samples at different *postmortem* intervals (PMIs), from the 5th to 60th day of exhumation, C) Mean and standard deviation of the percent loss of coat area among the rabbit, cattle, and pig ear samples at different *postmortem* intervals (PMIs), from the 5th to 60th day of exhumation. Means followed by different lower-case letters in the line are different by the Tukey test (p<0,05), C') Graphic characterization of the percent loss of coat area among the rabbit, cattle, and pig ear samples at different *postmortem* intervals (PMIs), from the 5th to 60th day of exhumation.

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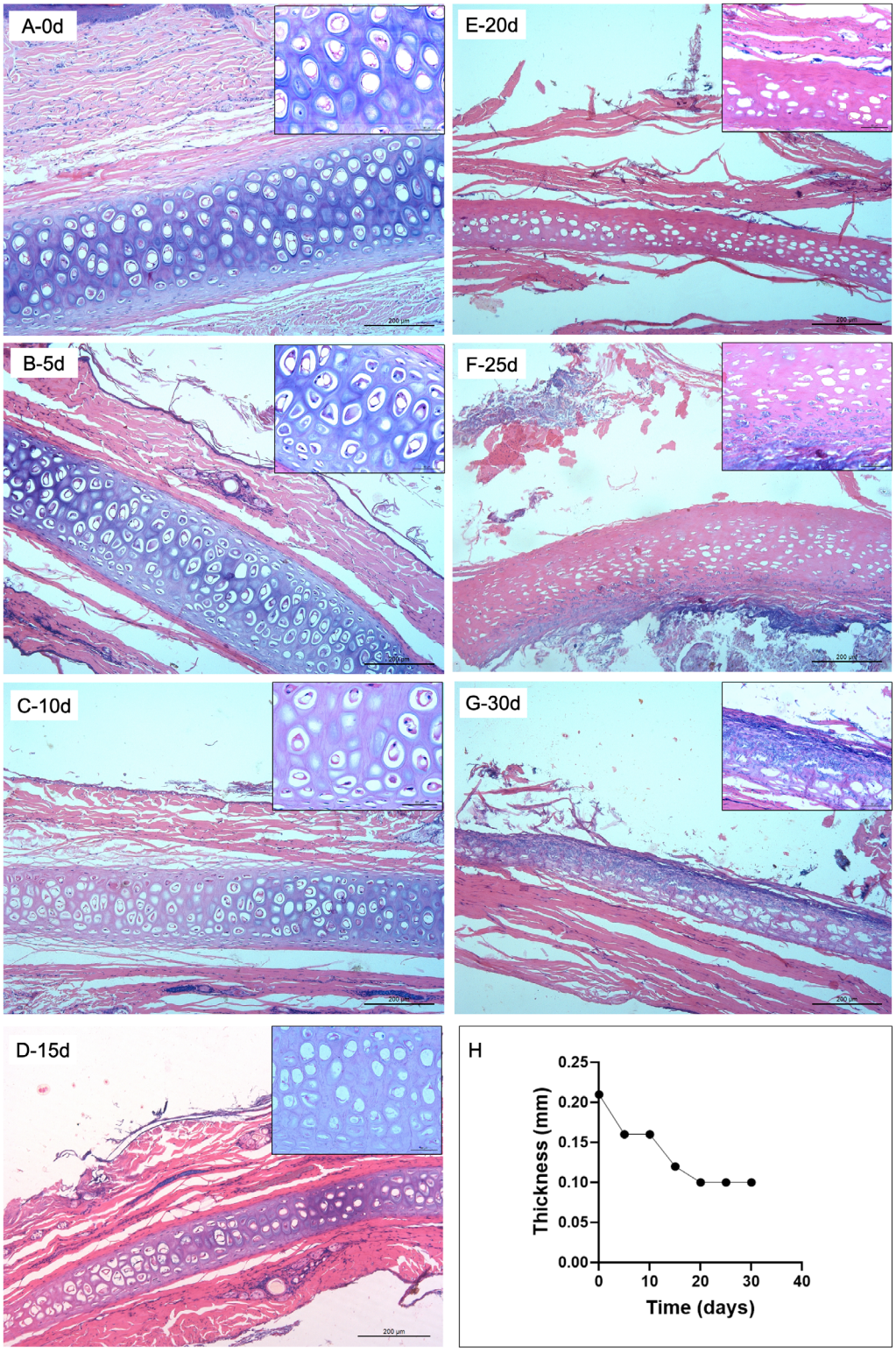

Figure 5. Microscopic *postmortem* changes in the rabbit ear samples during different *postmortem* intervals (PMIs): A) Day 0 with no *postmortem* changes, B) day 5 with partial loss of cartilage tinctorial affinity and chondrocytes with nuclei (detail), C) day 10, D) day 15 with thinned cartilage and partial loss of tinctorial affinity and chondrocyte nuclei (detail), E) day 20 with total loss of cartilage tinctorial affinity and chondrocyte nuclei (detail), F) day 25 total loss of cartilage tinctorial affinity and chondrocyte nuclei and profuse bacterial and fungal colonization of the cartilage (detail), G) day 30 with marked cartilage thinning, loss of tissue architecture, and bacterial and fungal cartilage colonization (detail), H) Reduction in cartilage thickness as a function of PMI. Hematoxylin–eosin, Bar=200 µm.

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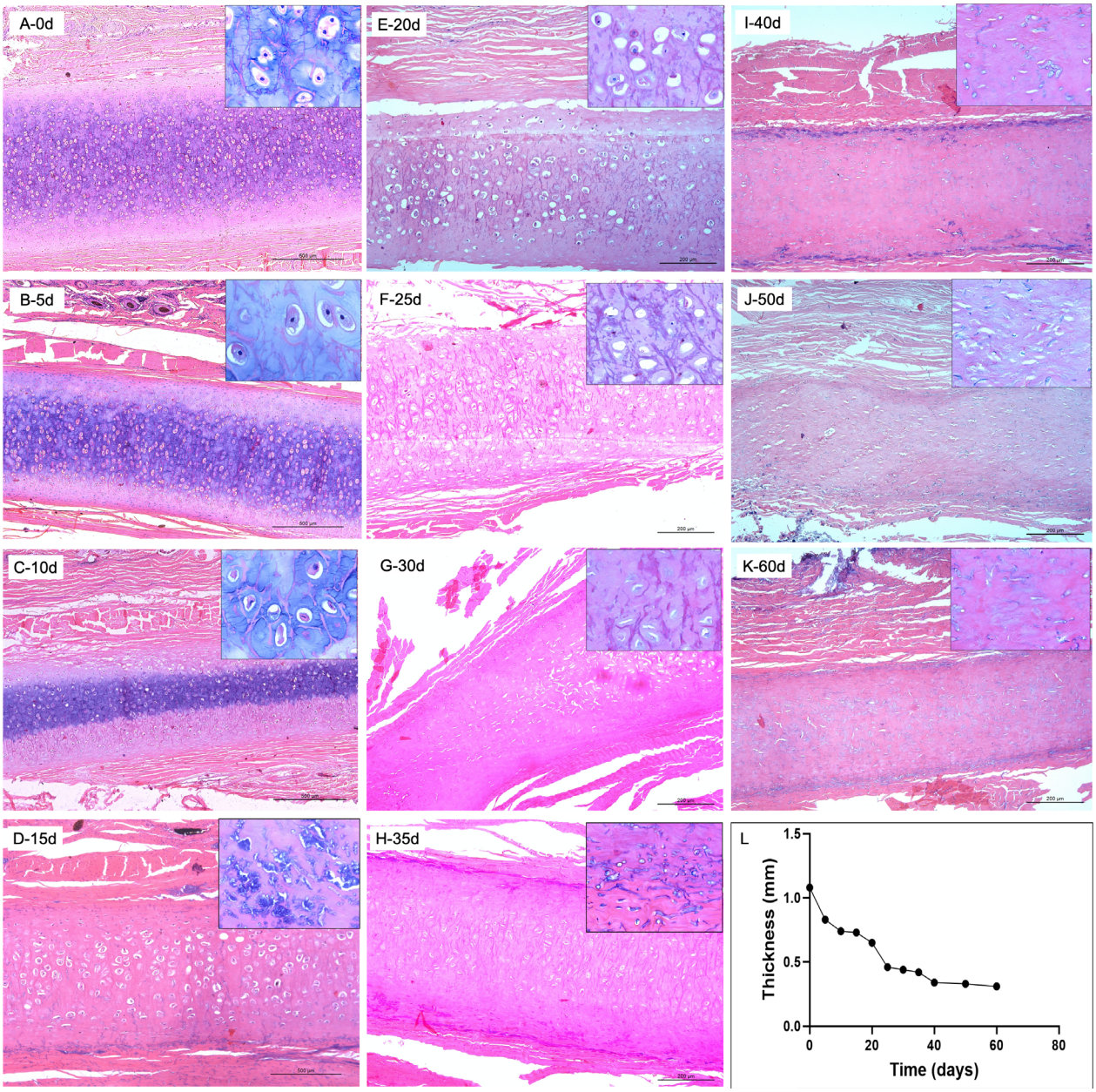

Figure 6. Microscopic *postmortem* changes in the cattle ear samples during different *postmortem* intervals (PMIs): A) Day 0 with no *postmortem* changes, B) day 5 with partial loss of cartilage tinctorial affinity and chondrocytes with nuclei (detail), C) day 10 with partial loss of cartilage tinctorial affinity and chondrocytes with nuclei (detail), D) day 15 with total loss of cartilage tinctorial affinity and profuse bacterial and fungal colonization of the adjacent tissues (detail), E) day 20 with total loss of cartilage tinctorial affinity and partial loss of chondrocyte nuclei (detail), F) day 25 with total loss of cartilage tinctorial affinity and chondrocyte nuclei (detail), G) day 30 with total loss of tissue architecture and chondrocyte nuclei (detail), H) day 35 with total loss of tissue architecture and fungal hyphae (detail), I) day 40, J) day 50, K) day 60 with total loss of tissue architecture and numerous fungal hyphae (detail), L) Reduction in cartilage thickness as a function of PMI . Hematoxylin–eosin, Bar=200 µm.

architecture. On the 40th day, bacteria and fungi were present in the cartilage in 75.00% and 37.5% of the samples, respectively. On the 50th day, bacteria were observed only in the cartilage (8.33%), and fungi were present in the tissue adjacent to the cartilage and in the cartilage in 16.66% and 33.33% of the samples, respectively.

On the 60th day, all samples showed total impairment of tissue architecture. Consequently, no sample showed tinctorial affinity or nuclear material. Colonization by fungi and bacteria was observed. Bacteria were present in the adjacent tissues and cartilage in 37.50% and 20.83% of the samples, respectively. Fungi were also observed in the

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connective and cartilage tissue in 37.5% and 54.16% of the samples, respectively.

The auricular cartilage thickness was significantly reduced on the 5th day (0.83 mm) compared with the value on day 0 (1.08 mm). Although the thickness reduced from the 10th to 20th days, the reduction was significant only on the 25th day (Fig. 6L).

Microscopic postmortem changes in pig ears

Microscopic *postmortem* findings of the pig ear samples at different PMIs are shown in Figure 7.

On day 0, all pig ear samples were completely preserved, covered by intact hairy skin and with perichondrium and cartilage free from any *postmortem* change. The cartilage

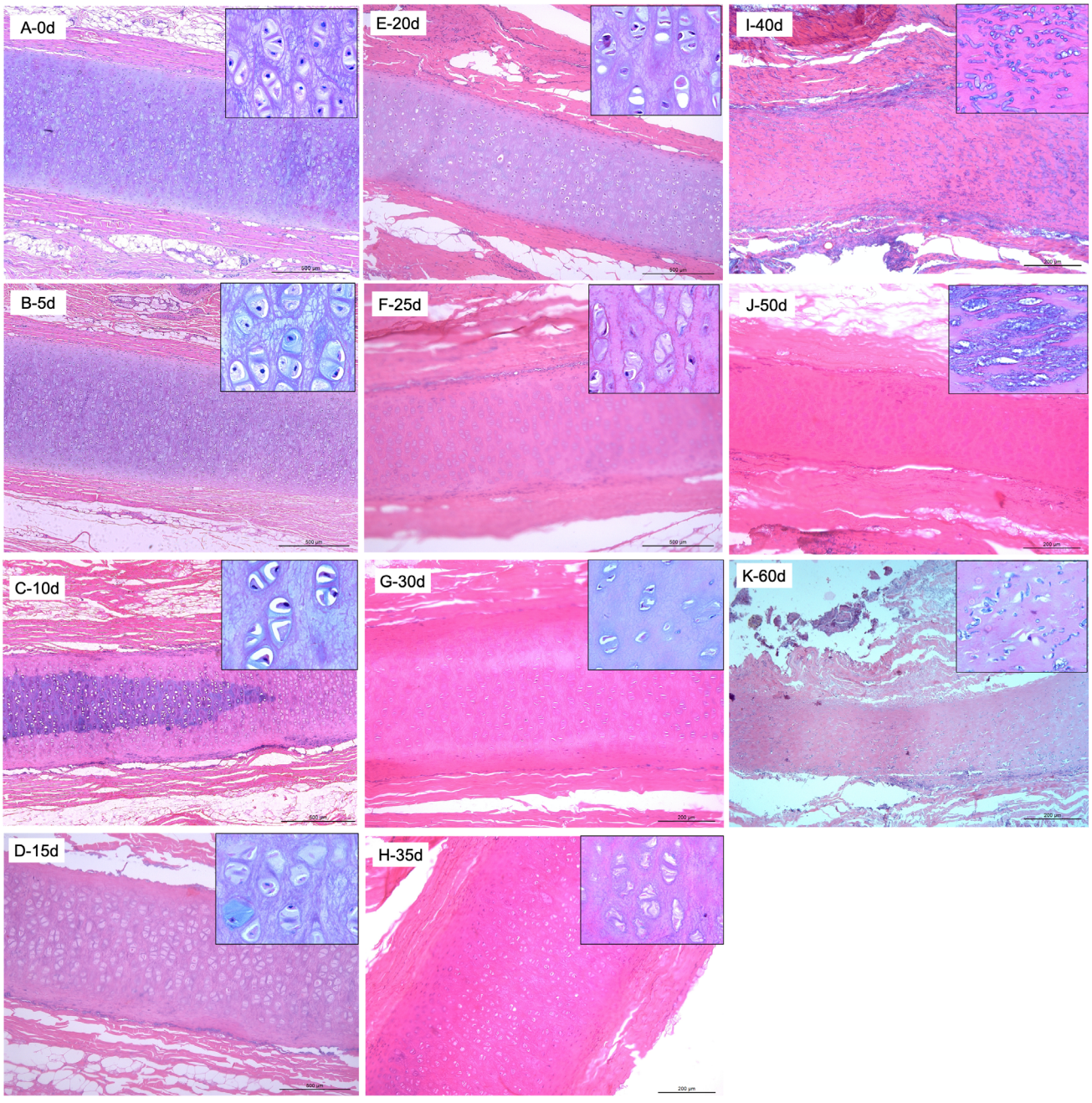

Figure 7. Microscopic *postmortem* changes in the pig ear samples during different *postmortem* intervals (PMIs): A) Day 0 with no *postmortem* changes, B) day 5, C) day 10 with partial loss of cartilage tinctorial affinity and chondrocytes with nuclei (detail), D) day 15, E) day 20, F) day 25, G) day 30 with total loss of cartilage tinctorial affinity and slight loss of chondrocyte nuclei (detail), H) day 25 with total loss of cartilage tinctorial affinity and chondrocyte nuclei (detail), I) day 40, J) day 50, K) day 60 with total loss of tissue architecture and fungal cartilage colonization (detail), L) Reduction in cartilage thickness as a function of PMI. Hematoxylin–eosin, Bar=200 µm.

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was intact and well stained with hematoxylin. Cartilage in the ping samples (1.04 mm) was approximately five times thicker than that in the rabbit samples but similar in thickness to that in the cattle samples. Chondrocyte gaps lacunae were much smaller than those in the rabbit samples, and chondrocytes were mononuclear.

On the 5th day, partial loss of cartilage tinctorial affinity was observed in 66.67% of the samples, and colonies of coccoid bacteria, similar to those detected in the other two species, were observed in the skin, adjacent tissues, and cartilage of 4.16% of the samples. Septate fungal hyphae, with negative images and parallel walls were observed in the skin and cartilage tissue of 4.16% of the samples.

On the 10th day, the percentage of samples with partial loss of cartilage tinctorial affinity increased to 75%, whereas 16.67% of the samples already showed total loss of color. Bacteria were observed in the skin (8.33%) and cartilage (33.33%). Fungal structures were not observed in any sample.

On the 15th day, majority of the samples showed total loss of cartilage tinctorial affinity (58.33%). Partial and total loss the chondrocyte nuclei and tissue architecture were observed in 25% and 20.83% of the samples, respectively. Bacterial colonies were observed in the skin and connective tissue and in the cartilage in 75% and 83.33% of the samples, respectively. Fungal structures were visualized once again in the skin and adjacent tissues in 16.66% of the samples and in the cartilage in 4.16% of the samples.

On the 20th day, total loss of cartilage tinctorial affinity was observed in 75% of the fragments. Similar to the observation of the 15th day, partial and total loss of chondrocyte nuclei and tissue architecture was observed in 25% and 20.83% of the samples, respectively. Bacteria were observed in the skin (20.83%) and cartilage (87.50%). Fungi were also observed in the skin and connective tissue (8.33%) as well as in the cartilage (25.0%).

On the 25th day, the proportion of samples showing total loss of cartilage tinctorial affinity, chondrocyte nuclear material, and tissue architecture increased to 83.33%, 66.67%, and 58.33%, respectively. Regarding microbial colonization, 91.66% of the samples harbored bacteria and 33.33% harbored fungi (33.33%) in the cartilage.

On the 30th, 35th, and 40th days, almost all samples (95.83%) showed complete loss of cartilage tinctorial affinity. Total loss of chondrocyte nuclei was observed in 66.7%–75% of the samples. Total impairment of tissue architecture increased over, with respectively 58.33% and 95.83% of the samples showing impairment at 30 and 40 days. Bacterial and fungal colonization varied widely among the PMIs. However, fungal colonization of the cartilaginous tissue was observed in 66.67% of the samples on the 40th day.

Finally, on the 50th and 60th days, all samples had completely lost tissue architecture and, thus, cartilage tinctorial affinity and chondrocyte nuclear material. On the 50th and 60th days, bacteria were predominantly observed in the cartilage (95.83% and 87.50%, respectively) and fungi in the adjacent connective tissue (87.50% and 62.50%, respectively).

Auricular cartilage thickness was significantly reduced only on the 15th day (0.79 mm) compared with the value on day 0 (1.04 mm). Although the thickness reduced from the 15th to 30th day, the reduction was significant only on the 30th day (0.42 mm) compared with the value on the 15th day. Thereafter, cartilage thickness continued to reduce, but the change was not statistically significant compared with the value on the 30th day. On the 60th day, the cartilage thickness was similar between the pig (0.28 mm) and cattle samples (Fig. 7L).

Correlation of macro- and microscopic variables with time

Based on the R^2 values for the rabbit (R^2=88.4), cattle (R^2= 87.1), and pig (R^2=94.3) samples, the correlation between time and percentage weight loss was considered strong.

Based on the R^2 values for the rabbit (R^2=56.5), cattle (R^2= 33.3), and pig (R^2=59.8) sample, the correlation between time and percentage tissue area loss was moderate for rabbits and pigs and weak for cattle.

Based on the R^2 values for the rabbit (R^2=37.8), cattle (R^2=59.8), and pig (R^2=52.9) samples, the correlation between time and percentage coat area loss was moderate for cattle and pigs and weak for rabbits.

Based on the R^2 values for the rabbit (R^2=63.4), cattle (R^2= 78.4), and pig (R^2=70.3) samples, the correlation between time and cartilage thickness was strong.

Discussion

The present study demonstrated that macro- and microscopic analysis of the auricular cartilage, specifically the analysis of percentage weight loss of the sample and cartilage thickness, can be used to estimate PMI, even in the cases of late *postmortem* decomposition and that the appearance and progression of the macro- and microscopic *postmortem* change of the auricular cartilage occur as a function of time and vary among samples of the same species at different time points as well as between different species.

Due to the effects of various soil conditions on the decomposition process and, consequently, on PMI estimation (10,22), soil physicochemical properties were analyzed in the present study. Soil pH, moisture, and temperature were measured daily. Based on the results, the soil was characterized as clayey–sandy with alkaline pH. However, this study did not aim to compare the effects of different types of soil on the decomposition of ear cartilage. Therefore, all samples were buried at a single location under the same soil. However, the rate of decomposition in clayey–sandy soils is faster than that in sandy soils, because the clay helps in retaining moisture, making the soil more humid and thus accelerating *postmortem* tissue degradation (22).

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Soil pH, measured at a depth similar to that of the pits, did not vary throughout the experimental period. This constancy in soil pH may be attributed to the characteristics of the tissue used in the present study. During cadaveric decomposition, several biochemical reactions occur. In the first two 2 weeks, there is a tendency for soil pH to increase at the burial site, due to the decomposition of nitrogenous residues contained in tissue organic matter, mainly proteins, releasing ammonia (NH_3). The NH_3 molecule is also further converted to NH_4^+, which in turn increases pH to alkaline. After 3 weeks, NH_3 is oxidized to nitrite (NO_2) and nitrate (NO_3^-), that reduce soil pH (10, 14).

Ear samples are small and composed of cartilaginous tissue and skin, with little organic matter; therefore, the formation and release of alkalizing and acidifying molecules were probably not sufficient to modify soil pH. In a previous study by Haslam and Tibbett (10), skeletal striated muscle samples were buried in alkaline, basic, or acidic soils. The authors observed increase in pH in the first week, followed by decrease in the third week of PMI. The authors argued that because muscle tissue is a major source of protein, significant amounts of nitrogen were released during decomposition, justifying the changes in soil pH (10). Likewise, Hopkins et al (22) reported increase in pH in cemetery soils due to the release of high amounts of NH_3 and its subsequent conversion to NH_4^+ from buried corpses.

Furthermore, in the present study, soil pH was classified as neutral. After 30 days of PMI, the samples of ear cartilage from rabbits showed marked changes, although none had completely decomposed. This observation differs from the finding reported by Paulis et al (15) that the rabbit ear samples buried in acidic soil (pH=5.5) did not remain viable for analysis after 30 days. Similarly, Haslam and Tibbett (10) reported that after 21 days of PMI, skeletal muscle tissues of sheep buried in acidic soil showed greater decomposition, followed by those buried in basic and alkaline soils.

In the present study, the percentage weight loss of samples progressively decreased and was strongly correlated with time in all three species. The percentage weight loss of the samples over time was expected due to the initial dehydration of soft tissues, which is responsible for significant loss in the mass of cadavers. In addition to water loss, tissue biodegradation reduces sample area. Because these two variables are correlated, samples weight decreases with PMI advancement due to reduction in their area (3,10).

Additionally, the percentage of tissue area lost decreased with increasing PMI, although its correlation with time was not strong. The reduction in sample area, in addition to weight loss, can also be explained by the decomposition process, through which tissues degrade over time and are reduced into smaller fragments (3). The slight loss of tissue throughout most of the analyzed PMIs proves that the auricular cartilage is resistant to the decomposition process after being buried. According to Alibegović et al (1), the avascular nature of the cartilage, abundance of ECM and collagen fibers, resistance of chondrocytes to acidosis and hypoxia increase the resistance of cartilage to *postmortem* decomposition. The rabbit cartilage samples were thinner than the cattle and pig samples; however, at 30 days of PMI, all samples still contained significant amount of tissue, even though 83.33% of the samples showed complete loss of cartilage architecture under microscopy.

Cadaveric wax or adipocere was first observed at 10 days in all three species. In rabbit samples, adipocere was observed only between the 20th and 25th days. In cattle samples, this change was observed until the 20th day. In pig and rabbit samples, no specific pattern was identified, but adipocere was observed on the 10th, 20th, and 35th days. Adipocere formation was expected because it is a common *postmortem* finding observed in buried corpses, resulting from the saponification of fat in anaerobic environments (26). The presence of cadaveric wax can help estimate PMI, since its formation occurs in the initial days after death; however, given the randomness at which it was observed in the present study, particularly in pig and rabbit samples, adipocere formation should not be used as the sole parameter.

Regardless of the animal species, the thickness of the ear cartilage progressively reduced with increasing PMI and was strongly correlated with time; therefore, auricular cartilage thickness can be considered a reliable parameter for PMI estimation.

Compared with the samples of the other two species, the rabbit samples exhibited reduced cartilage thickness. Specifically, on the 30th day, 37.5% of the rabbit samples had already lost between 50% and 100% of cartilage. Therefore, we anticipated that over 50% of the rabbit samples would not be able to resist decomposition after 150 days. Unexpectedly, however, all cattle samples had completely decomposed during the same period. This result is difficult to explain because the exhumation pits of all species were placed adjacent to one another and subjected to the same environmental conditions.

Nonetheless, differences in the biochemical composition of cartilage ECM among the three species may explain the faster decomposition of cattle samples. According to Chiu et al (7), unlike the glycosaminoglycan (GAG)-rich ear cartilage ECM of cattle, the ear cartilage ECM of rabbits contains trace amounts of GAGs. Furthermore, the early colonization of samples by microorganisms and the presence of arthropods and annelids in the pits must be considered. At the time of exhumation, arthropods and annelids were observed in the pits of rabbit samples at 30 day and of cattle samples at 20 and 40 days but not in the pits of pig samples. Although we could not ascertain whether arthropods and annelids were present before exhumation, these organisms are known to accelerate the rate of decomposition in buried corpses by consuming organic matter (mainly larvae) and increasing temperature (mainly in the intra-abdominal region) during organic matter consumption (21). Few forensic studies have reported the presence of arthropods or annelids at burial sites, but they did not establish the link between the activity of arthropods or annelids and the rate of tissue decomposition (20, 16).

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Histologically, the loss of cartilage tinctorial affinity started on the 5th day of PMI in the samples of all animal species, but all samples completely loss the cartilage tinctorial affinity at 30, 40, and 50 days in rabbits, cattle, and pigs, respectively. Such a complete loss of cartilage tinctorial affinity at more advanced PMI was expected, given the fact that collagen and elastin fibers of the cartilage ECM are degraded over time. However, our results differ from those reported by Rogers et al (18), who observed reduction in tinctorial affinity of pig articular cartilage only after 9 weeks, or 63 days, of exhumation. This discrepancy may be related to differences in the type of cartilage studied and the environmental conditions under which in the study was performed.

The loss of chondrocyte nuclear material and cartilage tissue architecture began on the 15th day of PMI in the samples of all animal species; however, at 30 days, this change was observed to the greatest extent in the rabbit samples, followed by the cattle and pig samples. On the 30th day of PMI, 83.33% of the rabbit samples had completely lost chondrocyte nuclei. As the rabbit samples were only observed until the 30th day, we could not determine the number of days required for all samples to show complete loss of chondrocyte nuclear material and cartilage tissue architecture. However, in the pig and cattle samples, total loss of chondrocyte nuclei and cartilage tissue architecture in 100% of the samples occurred at 50 and 60 days, respectively. In pig pelvic limbs, Rogers et al (18) observed the earliest partial loss of articular cartilage nuclei at 9 weeks (63 days) after exhumation (18). However, in their study, the joints were buried completely intact, with skin, musculature, tendons, and ligaments. Moreover, owing to the anatomy, the articular cartilage is less exposed to the environment than the auricular cartilage, which provides greater protection and resistance to the process of decomposition. In a study by Paulis et al (15), 20% of the rabbit ears had partially lost chondrocyte nuclei at 5 days of PMI and the cartilage turned unfeasible for analysis at 30 days. The faster rate of this change in Paulis et al (15) study than that observed in our present study can be explained by the differences in environmental and soil conditions.

In addition, bacterial colonization of samples was expected, since in the natural chronology of decomposition, following autolysis, heterolysis occurs, which is a transformative process occurring in the cadaver resulting from the action of microorganisms (3). However, the present study did not aim to identify the genera of bacteria and fungi involved. Rogers et al (17) isolated bacteria of the genus *Comamonas* sp. from the synovial fluid of the metatarsophalangeal joints of pigs, goats, and cattle after 3 days of inhumation (17). Among fungi, *Yarrowia lipolytica* has been reported to be the predominant species detected throughout the period of decomposition of pig carcasses exposed to open environments (9). However, studies evaluating cartilage tissues at different PMIs have not reported the presence of fungi (17,18,15). In the present study, differences among the animal species in terms of microbial colonization of the samples may explain the differences in their total decomposition time, particularly when comparing the rabbit samples with the cattle samples. The types and numbers of microorganisms involved in decomposition may explain the complete decomposition of all cattle samples within 150 days, unlike the rabbit samples.

Conclusions

Macro- and microscopic analysis of the auricular cartilage of rabbits, cattle, and pigs can be used to estimate PMI, even in the cases of late *postmortem* decomposition.

The mean percentage weight loss of the rabbit ear samples was greater than that of the cattle and pig ear samples for up to 30 days of PMI. At 60 days of PMI, the weight loss of the cattle and pig samples was comparable. Sample weight loss was strongly correlated with time in all three animal species, demonstrating that sample weight loss is a reliable parameter for estimating PMI.

The rabbit samples showed greater and earlier loss of coat area than the cattle and pig samples. Between the 35th and 60th days of PMI, the percentage loss of the coat area of the cattle and pig samples was comparable.

The loss of cartilage tinctorial affinity started on the 5th day of PMI in the samples of all animal species studied; however, complete loss of cartilage tinctorial affinity in 100% of the samples occurred at 30, 40, and 50 days in rabbit, cattle, and pig samples, respectively. The loss of chondrocyte nuclear material and cartilage tissue architecture began on the 15th day of PMI in the samples of all three animal species; however, at 30 days, the highest number of rabbit samples showed these changes, followed by the cattle and pig samples.

The cattle and pig samples showed early colonization by fungi and bacteria on the 5th day of PMI, contrary to the rabbit samples, which were colonized by fungi and bacteria on the 10th and 15th day of PMI, respectively.

Auricular cartilage thickness progressively decreased in the samples of all three animal species over time, and this thickness was strongly correlated with time. Therefore, auricular cartilage thickness can serve as a reliable parameter to estimate PMI. Overall, complete decomposition occurred the earliest in the cattle samples, followed by the rabbit and pig samples.

Conflict of interest

The authors declare that they have no conflicts of interest with respect to their authorship or publication of this article.

Ethical approval

Ethical approval was not required because rabbit, cattle, and pig ears were obtained from slaughterhouses.

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